purification by the "affinity capture and proteolytic release strategy". Both, bdSUMO^{Mut1} and bdSENP1^{MutB} are part of our novel SUMOvera system, which is described in detail through the following sections. In addition, bdSUMO mutants 8, 10, 11, 12, 13, 14, 15 as well as bdSENP1 mutants G, H, and K are described as alternative parts of the system.

SUMMARY OF THE CLAIMED ASPECTS

[0013] In more generic terms, the present invention relates to a fusion protein, comprising the structure

$$N\text{-}PCS^{Y}\text{-}degSig_{N}\text{-}M\text{-}PCS^{X}\text{-}degSig_{C}\text{-}C;$$

wherein N represents the N-terminus;

PCS^{Y} and PCS^{X} each represent a protease cleavage site (PCS), which differ from each other in at least one amino acid residue;

$degSig_{N}$ represents a degradation signal, which promotes degradation of the fusion protein in a host cell if PCS^{Y} is cleaved by a protease such that the first residue of $degSig_{N}$ becomes the new N-terminus of the remaining fusion;

M represents a cytoplasmic selection marker; and

$degSig_{C}$ represents a second degradation signal, which promotes degradation of the fusion protein in a host cell if PCS^{X} is not cleaved by a protease; and

C represents the C-terminus.

[0014] Further provided is a nucleic acid construct, comprising a nucleic acid sequence coding for the fusion protein of the present disclosure.

[0015] Also provided is a nucleic acid expression construct library, comprising a plurality of diversified nucleic acid expression constructs of the present disclosure, wherein the nucleic acid encoding PCS^{Y} of the fusion protein comprises a diversity such that in the encoded PCS^{Y} at least one amino acid position is diversified.

[0016] The present disclosure moreover provides a plurality of host cells, wherein each member of the plurality of host cells comprises a nucleic acid expression construct of the present disclosure, which is not diversified, or a member of a plurality of diversified nucleic acid expression constructs according to the present disclosure, wherein the host cells promote degradation the fusion protein via $degSig_{N}$, if PCS^{Y} is cleaved by a protease, and promote degradation of the fusion protein via $degSig_{C}$, if PCS^{X} is not cleaved by a protease. In embodiments, the host cells are capable of simultaneously expressing a protease of interest and the fusion protein encoded by the nucleic acid expression construct, wherein said protease of interest is capable of cleaving PCS^{X}.

[0017] Alternatively, the host cells may comprise a first non-diversified nucleic acid expression construct according to the present disclosure, and each member of said plurality of host cells comprises a member of a plurality of second expression constructs encoding a diversified protease of interest, wherein the host cells are capable of simultaneously expressing said diversified protease of interest together with the fusion protein encoded by said first expression construct, wherein said plurality of second expression constructs is derived from a protease capable of cleaving PCS^{Y} of the fusion protein of the first expression construct, and whereby the plurality of second expression constructs comprises a diversity in at least one amino acid position at the protease interface interacting with said PCS^{Y}.

[0018] Furthermore, the present disclosure provides a method for simultaneously testing whether (a) a first protease cleavage site PCS^{Y} is not cleaved by a protease of interest, and (b) whether a second protease cleavage site PCS^{X} is cleaved by said protease of interest, comprising the steps of

[0019] (i) providing a host cell comprising a first (non-diversified) nucleic acid construct according to the present disclosure and a second expression construct for expression of a protease of interest, wherein the host cell is capable of simultaneously expressing the fusion protein and said protease of interest, and wherein the host cell promotes degradation of the fusion protein via $degSig_{N}$, if PCS^{Y} is cleaved by a protease; and promotes degradation of the fusion protein via $degSig_{C}$, if PCS^{X} is not cleaved by a protease;

[0020] (ii) cultivating the host cell of step (i) under conditions such that the fusion protein and the protease of interest are simultaneously expressed; and

[0021] (iii) subjecting the host cell of step (ii) to selective conditions using the cognate selecting agent for the selection marker of the fusion protein encoded by the first nucleic acid construct;

wherein growth of the host cell in the presence of the selective conditions applied in step (iii) indicates that the first protease cleavage site PCS^{Y} is not cleaved by said protease of interest, and that said second protease cleavage site PCS^{X} is cleaved by said protease of interest of said second nucleic acid expression construct; preferably wherein the selection marker confers antibiotic resistance to the host cell.

[0022] Additionally, the present disclosure provides a method for identifying a protease cleavage site variant PCS^{Y} of a first protease cleavage site PCS^{X}, wherein PCS^{Y} is not cleaved by a protease of interest, comprising the steps of

[0023] (i) providing a plurality of host cells, wherein each member of said plurality of host cells comprises a member of a plurality of first nucleic acid constructs according to the present disclosure, which encodes for a diversified variant PCS^{Y} of a first protease cleavage site PCS^{X}, and a second expression construct for expression of a protease of interest, wherein said protease of interest is capable of cleaving PCS^{X}, whereby the plurality of host cells is capable of simultaneously expressing the fusion protein encoded by the first nucleic acid construct and said protease of interest, and wherein the host cells promote degradation of the fusion protein via $degSig_{N}$, if PCS^{Y} is cleaved by a protease; and promote degradation of the fusion protein via $degSig_{C}$, if PCS^{X} is not cleaved by a protease;

[0024] (ii) cultivating the plurality of host cells of step (i) under conditions such that the fusion protein and the protease of interest are simultaneously expressed; and

[0025] (iii) subjecting the plurality of host cells of step (ii) to selective conditions using the cognate selecting agent for the selection marker of the fusion protein encoded by the plurality of first nucleic acid constructs;

[0026] (iv) identifying a host cell, which has been positively selected in step (iii), and identifying the sequence of PCS^{Y} of the first nucleic acid construct of the identified host cell, wherein PCS^{Y} is a protease cleavage site variant of a first protease cleavage site PCS^{X}, and wherein PCS^{Y} is not cleaved by said protease of interest of the second expression construct.